

exemplary classes of agents were identified: agents that alter endocytosis, e.g., a compound of formula (III) (dependent claim 47), endosomal processing, e.g., a compound of formula(I)-(II) (dependent claim 29 and dependent claim 37, respectively), or ubiquitination, e.g., a compound of formula (IV) (dependent claim 81) (page 4, lines 29-31 and page 8, lines 7-21). Note that formulas (I), (II) and (IV) are peptidic compounds. Moreover, formula (I) and formula (II) are tripeptidic compounds with structural similarities. For instance, formula (I) and formula (II) are based on a tripeptidic structure with an N-terminal blocking group and a formyl group at the C-terminus.

Based on the Applicant's disclosure, Applicant's Representatives propose the following groups of claims for election in a revised Restriction Requirement: claims 1-12, 29-46, and 79-84 (group A), directed to methods to identify agents that alter AAV transduction of mammalian cells with a peptidic agent; claims 1-8, 10-12 and 47-78 (group B), directed to methods to identify agents that alter AAV transduction of mammalian cells with a non-peptidic agent; claims 13-46 and 79-84 (group C), directed to methods of using a peptidic agent to alter AAV transduction of a mammalian cell or expression of a transgene in a mammalian cell contacted with a recombinant AAV comprising the transgene; and claims 13-28 and 78-84 (group D), directed to methods of using a non-peptidic agent to alter AAV transduction of a mammalian cell or expression of a transgene in a mammalian cell contacted with a recombinant AAV comprising the transgene.

In response to the Restriction Requirement, Applicant provisionally elects, with traverse, the claims of Group I (claims 1-12, 29-36, and 83-84) directed to a method to identify an agent that alters AAV transduction of a mammalian cell, wherein the agent is a compound of formula (I). With regard to the election of species, Applicant provisionally elects, with traverse, the species of formula (I):  $R_1-A-(B)_n-C$ , wherein  $R_1$  is benzylocarbonyl, A and B are leucine,  $n = 0$  and C is norvalinal (N-carbobenzoxyl-L-leuciny-L-leuciny-L-norvalinal, Z-LLL). Note this species is encompassed by formula (I) and formula (II). Claims 1, 6-9, 13-18, 25-29, 31-32, 34-35, 37, and 39-46 read on Z-LLL. Reconsideration and withdrawal of the Restriction Requirement and the election of species, in view of the remarks below, is respectfully requested.

The Restriction Requirement is traversed on the basis that the inventions are so closely related within the context of the disclosure of the application that they cannot properly be

considered independent and distinct within the statutory meaning of 35 U.S.C. § 121. Claims directed to a method to identify agents of formula (I) that alter AAV transduction of mammalian cells (claims 1-12, 29-36, and 83-84; Group I) are clearly related to claims directed to methods to identify other agents that alter AAV transduction of mammalian cells (e.g., claims 1-12 and 37-84; Groups II-IV); and to claims directed to the use of those agents to enhance AAV transduction of mammalian cells or expression of a transgene delivered by a recombinant AAV to mammalian cells (claims 13-84; Groups V-XII).

In particular, claims directed to a method to identify agents of formula (I) that alter AAV transduction of mammalian cells (claims 1-12, 29-36, and 83-84; Group I) are clearly related to claims directed to a method to identify agents of formula (II) that alter AAV transduction of mammalian cells (claims 1-12, 37-46, and 83-84; Group II); claims directed to methods of using an agent of formula (I) to alter AAV transduction of mammalian lung cells or expression of a transgene delivered by a recombinant AAV to mammalian lung cells (claims 13, 15, 17-27, 29-36, and 83-84; Group V); claims directed to methods of using an agent of formula (II) to alter AAV transduction of mammalian lung cells or expression of a transgene delivered by a recombinant AAV to mammalian lung cells (claims 13, 15, 17-27, 37-46, and 83-84; Group VI); claims directed to methods of using an agent of formula (I) to alter AAV transduction of mammalian liver cells or expression of a transgene delivered by a recombinant AAV to mammalian liver cells (claims 14, 16-26, 28-36, and 83-84; Group IX); and claims directed to methods of using an agent of formula (II) to alter AAV transduction of mammalian liver cells or expression of a transgene delivered by a recombinant AAV to mammalian liver cells (claims 14, 16-26, 28, 37-46, and 83-84; Group X), as formula (I) and formula (II) have structural similarities.

The Restriction Requirement is also traversed on the basis that Restriction Requirements are optional in all cases. M.P.E.P. § 803. If the search and examination of an entire application can be made without serious burden, the Examiner must examine it on the merits, even though it arguably may include claims to distinct or independent inventions. M.P.E.P. § 803. Moreover, it is submitted that Applicant should not be required to incur the additional costs associated with the filing of multiple divisional applications in order to obtain protection for the claimed subject

matter. Due to the relatedness of the subject matter of the claims in Groups I-XII, for instance, Groups I-IV and VII-XII, the claims in Groups I-XII, e.g., Groups I-IV and VII-XII, can be efficiently and effectively searched in a single search with no additional burden placed on the Examiner. Evidence that the claims in at least Groups I-IV and VII-XII can be efficiently and effectively searched in a single search with no additional burden placed on the Examiner is provided in the Restriction Requirement as those claims are in the same class and subclass for search purposes. Moreover, the claims in Groups I-II, V-VI, and IX-X can be efficiently and effectively searched in a single search with no additional burden placed on the Examiner as a compound of formula (I) and a compound of formula (II) have structural similarities

Further, as claim 1 is a linking claim for claim 29 (a compound of formula (I)), claim 37 (a compound of formula (II)), claim 47 (a compound of formula (III)), and claim 81 (a compound of formula (IV)) and claims dependent on claims 29, 37, 47, and 81, claims 1-12 and 29-84 should be examined in the same application. M.P.E.P. 809.03. Thus, the Restriction Requirement is properly traversed. Accordingly, reconsideration and withdrawal of the Restriction Requirement is respectfully requested.

In the event the Examiner remains of the opinion that the restriction is proper as stated in the Restriction Requirement dated October 11, 2001, Applicant's Representatives respectfully request rejoinder of Groups II, V-VI and IX-X, or Groups V and IX, with Group I, upon a notice of allowable subject matter for the claims in Group I.

With respect to the requirement to elect species, the requirement is traversed on the basis that the species have a disclosed relationship. As discussed above, a compound of formula (I) (and a compound of formula (II)) inhibits endosomal processing. Thus, the requirement for an election of species is properly traversed and reconsideration is respectfully requested.

PRELIMINARY AMENDMENT AND RESPONSE TO RESTRICTION REQUIREMENT

Serial Number: 09/689,136

Filing Date: October 12, 2000

Title: COMPOUNDS AND METHODS TO ENHANCE rAAV TRANSDUCTION

Page 5

Dkt: 875.032US1

The Examiner is invited to contact Applicant's attorney (612-373-6959) if there are any questions concerning this Response or if prosecution of this application may be assisted thereby.

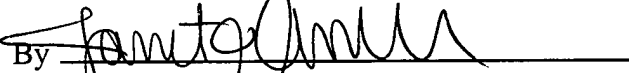
Respectfully submitted,

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Date April 11, 2002

By   
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CERTIFICATE UNDER 37 CFR 1.8: The undersigned hereby certifies that this correspondence is being deposited with the United States Postal Service with sufficient postage as first class mail, in an envelope addressed to: Commissioner of Patents, Washington, D.C. 20231, on this 11 day of April, 2002.

Janet E. Embretson  
Name

  
Signature

Docket No. 00875.032US1  
WD #435504

Client Reference Number N9-67

**Clean Version of Pending Claims**



**COMPOUNDS AND METHODS TO ENHANCE rAAV TRANSDUCTION**

Applicant: John F. Engelhardt et al.  
Serial No.: 09/689,136

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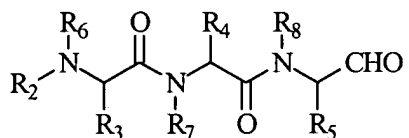
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1. A method to identify an agent that alters adeno-associated virus transduction of a mammalian cell, comprising:
  - a) contacting the mammalian cell with the agent and virus; and
  - b) detecting or determining whether virus transduction is altered.
2. The method of claim 1 wherein the cell is a mammalian lung cell.
3. The method of claim 1 wherein the cell is a mammalian liver cell.
4. The method of claim 1 wherein the cell is a human cell, canine cell, murine cell, rat cell or rabbit cell.
5. The method of claim 1 wherein the transduction is enhanced.
6. The method of claim 1 wherein endosomal processing is enhanced.
7. The method of claim 1 wherein the agent is an endosomal protease inhibitor.
8. The method of claim 7 wherein the agent is a cysteine protease inhibitor.
9. The method of claim 1 wherein the agent is a peptide or analog thereof.
10. The method of claim 1 wherein the virus is recombinant adeno-associated virus.
11. The method of claim 10 wherein the recombinant virus encodes a therapeutic peptide or polypeptide.
12. The method of claim 10 wherein the recombinant virus comprises a marker gene or a selectable gene.
13. A method to alter adeno-associated virus transduction of a mammalian lung cell, comprising: contacting the mammalian lung cell with an amount of an agent and an amount of virus effective to alter virus transduction.
14. A method to alter adeno-associated virus transduction of a mammalian liver cell, comprising: contacting the mammalian liver cell with an amount of an agent and an

amount of virus effective to alter virus transduction.

15. A method to alter the expression of a transgene in a mammalian lung cell, comprising: contacting the mammalian lung cell with an amount of an agent and an amount of recombinant adeno-associated virus comprising the transgene so as to alter expression of the transgene.
16. A method to alter the expression of a transgene in a mammalian liver cell, comprising: contacting the mammalian liver cell with an amount of an agent and an amount of recombinant adeno-associated virus comprising the transgene so as to alter expression of the transgene.
17. A method comprising: contacting a mammal subjected to gene therapy with recombinant adeno-associated virus comprising a transgene with an amount of an agent effective to alter expression of the transgene in the cells of the mammal.
18. The method of claim 13, 14, 15, 16, or 17 wherein endosomal processing of the virus is altered.
19. The method of claim 13 or 14 wherein the virus is recombinant adeno-associated virus.
20. The method of claim 19 wherein the recombinant virus encodes a therapeutic peptide or polypeptide.
21. The method of claim 13 or 14 wherein the recombinant virus encodes a therapeutic peptide or polypeptide.
22. The method of claim 15, 16, or 17 wherein the recombinant virus encodes a therapeutic peptide or polypeptide.
23. The method of claim 13, 14, 15, 16 or 17 wherein the cell is contacted with the agent before the cell is contacted with the virus.
24. The method of claim 13, 14, 15, 16 or 17 wherein the cell is contacted with the virus before the cell is contacted with the agent.
25. The method of claim 13, 14, 15, 16 or 17 wherein virus transduction is enhanced.
26. The method of claim 15, 16 or 17 wherein transgene expression is enhanced.

27. The method of claim 17 wherein expression is altered in lung cells.
28. The method of claim 17 wherein expression is altered in liver cells.
29. The method of claim 1, 13, 14, 15, 16 or 17 wherein the agent is a compound of formula (I):  $R_1-A-(B)_n-C$  wherein  $R_1$  is an N-terminal amino acid blocking group; each A and B is independently an amino acid; C is an amino acid wherein the terminal carboxy group has been replaced by a formyl (CHO) group; and n is 0, 1, 2, or 3; or a pharmaceutically acceptable salt thereof.
30. The method of claim 29 wherein  $R_1$  is  $(C_1-C_{10})$ alkanoyl.
31. The method of claim 29 wherein  $R_1$  is acetyl or benzyloxycarbonyl.
32. The method of claim 29 wherein each A and B is independently alanine, arginine, glycine, isoleucine, leucine, valine, nor-leucine or nor-valine.
33. The method of claim 29 wherein each A and B is isoleucine.
34. The method of claim 29 wherein C is alanine, arginine, glycine, isoleucine, leucine, valine, nor-leucine or nor-valine, wherein the terminal carboxy group has been replaced by a formyl (CHO) group.
35. The method of claim 29 wherein C is nor-leucine or nor-valine, wherein the terminal carboxy group has been replaced by a formyl (CHO) group.
36. The method of claim 29 wherein  $R_1$  is  $(C_1-C_{10})$ alkanoyl or benzyloxycarbonyl; A and B are each isoleucine; C is nor-leucine or nor-valine, wherein the terminal carboxy group has been replaced by a formyl (CHO) group; and N is 1.
37. The method of claim 1, 13, 14, 15, 16 or 17 wherein the agent is a compound of formula (II):



(II)

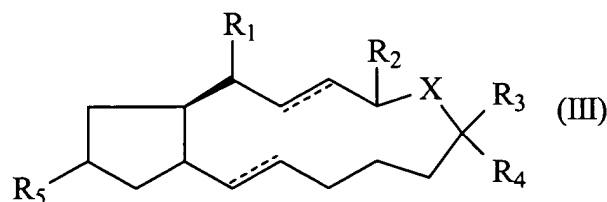
wherein

$R_2$  is an N-terminal amino acid blocking group;

$R_3$ ,  $R_4$ , and  $R_5$  are each independently hydrogen,  $(C_1-C_{10})$ alkyl, aryl or aryl $(C_1-C_{10})$ alkyl; and

$R_6$ ,  $R_7$ , and  $R_8$  are each independently hydrogen,  $(C_1-C_{10})$ alkyl, aryl or aryl $(C_1-C_{10})$ alkyl; or a pharmaceutically acceptable salt thereof.

38. The method of claim 37 wherein  $R_2$  is  $(C_1-C_{10})$ alkanoyl.
39. The method of claim 37 wherein  $R_2$  is acetyl or benzyloxycarbonyl.
40. The method of claim 37 wherein  $R_3$  is hydrogen or  $(C_1-C_{10})$ alkyl.
41. The method of claim 37 wherein  $R_3$  is 2-methylpropyl.
42. The method of claim 37 wherein  $R_4$  is hydrogen or  $(C_1-C_{10})$ alkyl.
43. The method of claim 37 wherein  $R_4$  is 2-methylpropyl.
44. The method of claim 37 wherein  $R_5$  is hydrogen or  $(C_1-C_{10})$ alkyl.
45. The method of claim 37 wherein  $R_5$  is butyl or propyl.
46. The method of claim 37 wherein  $R_2$  is acetyl or benzyloxycarbonyl;  $R_3$  and  $R_4$  are each 2-methylpropyl;  $R_5$  is butyl or propyl; and  $R_6$ ,  $R_7$ , and  $R_8$  are each independently hydrogen.
47. The method of claim 1, 13, 14, 15, 16 or 17 wherein the agent is a compound of formula (III):  
wherein



$R_1$  is H, halogen,  $(C_1-C_{10})$ alkyl,  $(C_1-C_{10})$ alkenyl,  $(C_1-C_{10})$ alkynyl,  $(C_1-C_{10})$ alkoxy,  $(C_1-C_{10})$ alkanoyl,  $(=O)$ ,  $(=S)$ , OH, SR, CN,  $NO_2$ , trifluoromethyl or  $(C_1-C_{10})$ alkoxy, wherein any alkyl, alkenyl, alkynyl, alkoxy or alkanoyl may optionally be substituted with one or more halogen, OH, SH, CN,  $NO_2$ , trifluoromethyl, NRR or SR, wherein each R is independently H or  $(C_1-C_{10})$ alkyl;

$R_2$  is  $(=O)$  or  $(=S)$ ;



R<sub>3</sub> is H, (C<sub>1</sub>-C<sub>10</sub>)alkyl, (C<sub>1</sub>-C<sub>10</sub>)alkenyl, (C<sub>1</sub>-C<sub>10</sub>)alkynyl, (C<sub>1</sub>-C<sub>10</sub>)alkoxy or (C<sub>3</sub>-C<sub>8</sub>)cycloalkyl, wherein any alkyl, alkenyl, alkynyl, alkoxy or cycloalkyl may optionally be substituted with one or more halogen, OH, CN, NO<sub>2</sub>, trifluoromethyl, SR, or NRR, wherein each R is independently H or (C<sub>1</sub>-C<sub>10</sub>)alkyl;

R<sub>4</sub> is H, (C<sub>1</sub>-C<sub>10</sub>)alkyl, (C<sub>1</sub>-C<sub>10</sub>)alkenyl, (C<sub>1</sub>-C<sub>10</sub>)alkynyl, (C<sub>1</sub>-C<sub>10</sub>)alkoxy or (C<sub>3</sub>-C<sub>8</sub>)cycloalkyl, wherein any alkyl, alkenyl, alkynyl, alkoxy or cycloalkyl may optionally be substituted with one or more halogen, OH, CN, NO<sub>2</sub>, trifluoromethyl, SR, or NRR, wherein each R is independently H or (C<sub>1</sub>-C<sub>10</sub>)alkyl;

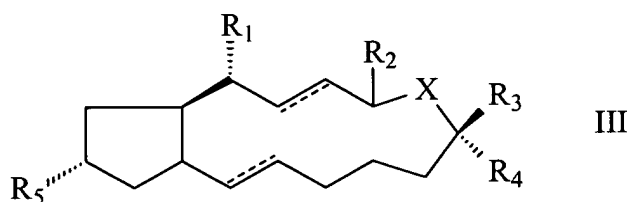
R<sub>5</sub> is H, halogen, (C<sub>1</sub>-C<sub>10</sub>)alkyl, (C<sub>1</sub>-C<sub>10</sub>)alkenyl, (C<sub>1</sub>-C<sub>10</sub>)alkynyl, (C<sub>1</sub>-C<sub>10</sub>)alkoxy, (C<sub>1</sub>-C<sub>10</sub>)alkanoyl, (=O), (=S), OH, SR, CN, NO<sub>2</sub> or trifluoromethyl, wherein any alkyl, alkenyl, alkynyl, alkoxy or alkanoyl may optionally be substituted with one or more halogen, OH, SH, CN, NO<sub>2</sub>, trifluoromethyl, NRR or SR, wherein each R is independently H or (C<sub>1</sub>-C<sub>10</sub>)alkyl; and

X is O, S or NR wherein R is H or (C<sub>1</sub>-C<sub>10</sub>)alkyl, or a pharmaceutically acceptable salt thereof.

48. The method of claim 47 wherein R<sub>1</sub> is halogen, CN, NO<sub>2</sub>, trifluoromethyl or OH.
49. The method of claim 47 wherein R<sub>1</sub> is OH.
50. The method of claim 47 wherein R<sub>2</sub> is (=O).
51. The method of claim 47 wherein R<sub>3</sub> is H or (C<sub>1</sub>-C<sub>10</sub>)alkyl.
52. The method of claim 47 wherein R<sub>3</sub> is methyl.
53. The method of claim 47 wherein R<sub>4</sub> is H or (C<sub>1</sub>-C<sub>10</sub>)alkyl.
54. The method of claim 47 wherein R<sub>4</sub> is H.
55. The method of claim 47 wherein R<sub>5</sub> is halogen, CN, NO<sub>2</sub>, trifluoromethyl or OH.
56. The method of claim 47 wherein R<sub>5</sub> is OH.
57. The method of claim 47 wherein X is O or S.
58. The method of claim 47 wherein X is O.
59. The method of claim 47 wherein both ----- are a single bond.
60. The method of claim 47 wherein one ----- is a double bond.

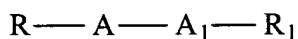
61. The method of claim 47 wherein both ----- are a double bond.
62. The method of claim 45 wherein  $R_1$  is OH,  $R_2$  is (=O),  $R_3$  is methyl,  $R_4$  is H,  $R_5$  is OH, X is O, and both ----- are a double bond.

63. (Amended) The method of claim 47 wherein the compound is a compound of formula (III):



64. The method of claim 63 wherein  $R_1$  is halogen, CN,  $\text{NO}_2$ , trifluoromethyl or OH.
65. The method of claim 63 wherein  $R_1$  is OH.
66. The method of claim 63 wherein  $R_2$  is (=O).
67. The method of claim 63 wherein  $R_3$  is H or  $(\text{C}_1\text{-C}_{10})$ alkyl.
68. The method of claim 63 wherein  $R_3$  is methyl.
69. The method of claim 63 wherein  $R_4$  is H or  $(\text{C}_1\text{-C}_{10})$ alkyl.
70. The method of claim 63 wherein  $R_4$  is H.
71. The method of claim 63 wherein  $R_5$  is halogen, CN,  $\text{NO}_2$ , trifluoromethyl or OH.
72. The method of claim 63 wherein  $R_5$  is OH.
73. The method of claim 63 wherein X is O or S.
74. The method of claim 63 wherein X is O.
75. The method of claim 63 wherein both ----- are a single bond.

76. The method of claim 63 wherein one ----- is a double bond.
77. The method of claim 63 wherein both ----- are a double bond.
78. The method of claim 63 wherein  $R_1$  is OH,  $R_2$  is (=O),  $R_3$  is methyl,  $R_4$  is H,  $R_5$  is OH, X is O, and both ----- are a double bond.
79. The method of claim 1, 13, 14, 15, 15 or 17 wherein the agent inhibits the activation of ubiquitin, the transfer of ubiquitin to the ubiquitin carrier protein, ubiquitin ligase, or a combination thereof.
80. The method of claim 1, 13, 14, 15, 15 or 17 wherein the agent inhibits ubiquitin ligase.
81. The method of claim 1, 13, 14, 15, 15 or 17 wherein the agent is a compound of formula (IV):



wherein R is hydrogen, an amino acid, or a peptide, wherein the N-terminus amino acid can optionally be protected at the amino group with acetyl, acyl, trifluoroacetyl, or benzyloxycarbonyl; A is an amino acid or a direct bond;  $A_1$  is an amino acid; and  $R_1$  is hydroxy or an amino acid, wherein the C-terminus amino acid can optionally be protected at the carboxy group with  $(C_1-C_6)$ alkyl, phenyl, benzyl ester or amide (e.g.,  $C(=O)NR_2$ , wherein each R is independently hydrogen or  $(C_1-C_6)$ alkyl); or a pharmaceutically acceptable salt thereof.

82. The method of claim 81 wherein the agent is H-Leu-Ala-OH, H-His-Ala-OH, or a combination thereof.
83. The method of claim 1, 13, 14, 15, 15 or 17 further comprising administering a second agent that enhances the activity of the agent.
84. The method of claim 83 wherein the second agent is EGTA.